PB82-234303

Short-Term Screening Test for Quick Responses to Pollutants Where Health Effects Data Are Lacking

IIT Research Inst.
Chicago, IL

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

Feb 82

U.S. Department of Commerce
National Technical Information Service
NTIS

PB82-234303

EPA 600/1-82-004
February 1982

SHORT-TERM SCREENING TEST FOR QUICK RESPONSES TO POLLUTANTS WHERE HEALTH EFFECTS DATA ARE LACKING

by

John G. Drummond
James D. Fenters
Richard Ehrlich
Catherine Aranyi
Leonard J. Schiff

IIT Research Institute
Life Sciences Research Division
Chicago, Illinois 60616

Contract No. 68-02-2088

Project Officer

Dr. Judith Graham
Inhalation Toxicology Branch
Environmental Toxicology Division
Health Effects Research Laboratory
US Environmental Protection Agency

OFFICE OF RESEARCH AND DEVELOPMENT
HEALTH EFFECTS RESEARCH LABORATORY
US ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NC 27711

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/1-82-004	ORD Report	PB82 234303

4. TITLE AND SUBTITLE	5. REPORT DATE
Short-term Screening Test for Quick Responses to Pollutants Where Health Effects Data Are Lacking	February 1982
	6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
John G. Drummond, James D. Fenters, Richard Ehrlich, Catherine Aranyi, and Leonard J. Schiff	

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
IIT Research Institute Life Sciences Research Division Chicago, Illinois 60616	C9GA1A
	11. CONTRACT/GRANT NO.
	Contract No: 68-02-2088

12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Office of Research and Development (RTP,NC) Health Effects Research Laboratory US Environmental Protection Agency Research Triangle Park, NC 27711	14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES

Project Officer: Dr. Judith A. Graham Telephone: (919) 541-2531

16. ABSTRACT

Results of assessment of various health effects parameters were correlated to determine which tests were the most sensitive indicators of toxic effects of exposure to sulfate aerosols. Inhalation studies were conducted involving single 3-hr exposure to various concentrations of cupric sulfate (0.43 - 2.64 mg/m^3), aluminum sulfate (1.65 - 2.75 mg/m^3), and aluminum ammonium sulfate (1.47 - 3.81 mg/m^3); and multiple 5 or 10 daily 3-hr exposures to cupric sulfate (0.1 mg/m^3). The test parameters studied in CD_1 mice were changes in mortality after respiratory infection with *Streptococcus pyogenes*; pulmonary bactericidal activity; pulmonary cell number, type, viability, and ATP content; and pulmonary morphology by scanning electron microscopy. Tracheal ciliary beating frequency and morphology were also studied in both CD_1 mice and Syrian golden hamsters.

Changes in mortality appeared to be the most sensitive and consistent indicators of pollutant damage. The other parameters produced evidence of damage but generally only at the higher pollutant concentrations. Cupric sulfate was the most toxic of the three sulfates but the differences in toxicity of the aluminum sulfate and aluminum ammonium sulfate were less clear. Between-sex differences in responses to the pollutants were evaluated.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	39
	20. SECURITY CLASS (This page)	22. PRICE
	UNCLASSIFIED	

DISCLAIMER

This report has been reviewed by the Health Effects Research Laboratory, US Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the US Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The complexities of environmental problems originate in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines. The Health Effects Research Laboratory conducts a coordinated environmental health research program in inhalation toxicology, genetic toxicology, neurotoxicology, developmental and experimental biology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, water pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides and other chemical pollutants. The Laboratory participates in and provides data for the development and revision of criteria documents on pollutants for which national ambient air quality and water quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of environmental regulatory decisions involving the protection of the health and welfare of all U.S. inhabitants.

The studies described in this report were designed to determine the ability of selected health effects parameters to provide accurate toxicity data on three particulate air pollutants: cupric sulfate, aluminum sulfate, and aluminum ammonium sulfate. The data were correlated to determine which parameters were the most sensitive indicators of toxic effects of the sulfates.

F. Gordon Hueter, Ph.D.
Director
Health Effects Research Laboratory

ABSTRACT

Results of assessment of various health-effects parameters were correlated to determine which tests were the most sensitive indicators of toxic effects of exposure to sulfate aerosols. Inhalation studies were conducted involving single 3-hr exposure to various concentrations of cupric sulfate (0.43-2.64 mg/m^3), aluminum sulfate (1.65-2.75 mg/m^3), and aluminum ammonium sulfate (1.47-3.81 mg/m^3); and multiple 5 or 10 daily 3-hr exposures to cupric sulfate (0.1 mg/m^3). The test parameters studied in CD_1 mice were changes in mortality after respiratory infection with *Streptococcus pyogenes*; pulmonary bactericidal activity; pulmonary cell number, type, viability, and ATP content; and pulmonary morphology by scanning electron microscopy. Tracheal ciliary beating frequency and morphology were also studied in both CD_1 mice and Syrian golden hamsters.

Changes in mortality appeared to be the most sensitive and consistent indicators of pollutant damage. The other parameters produced evidence of damage but generally only at the higher pollutant concentrations. Cupric sulfate was the most toxic of the three sulfates but the differences in toxicity of the aluminum sulfate and aluminum ammonium sulfate were less clear. Between-sex differences in response to the pollutants were evaluated.

CONTENTS

	Page
Foreword	iii
Abstract	iv
Figures	vi
Tables	vii
Abbreviations	viii
1. Introduction	1
2. Conclusions	2
3. Recommendations	3
4. Materials and Methods	4
Animals	4
Inhalation Exposures	4
Pollutants	4
Exposure Regimens	5
Experimental Parameters	6
Statistical Analyses	10
5. Results	11
Mortality	11
Pulmonary Defense Mechanisms	14
Tracheal Epithelium	18
Scanning Electron Microscopy	21
6. Discussion	27
References	31

FIGURES

Number		Page
1	Respiratory tract of mouse exposed to 2.53 mg/m^3 cupric sulfate for 3 hr	23
2	Respiratory tract of mouse exposed to 2.10 mg/m^3 aluminum ammonium sulfate for 3 hr	25
3	Respiratory tract of control mouse after 5 daily 3-hr exposures to filtered room air	25
4	Bronchus of mouse after 10 daily 3-hr exposures to 0.1 mg/m^3 cupric sulfate	26
5	Alveoli of mouse after 5 daily 3-hr exposures to 0.1 mg/m^3 cupric sulfate	26
6	Alveoli of mouse after 10 daily 3-hr exposures to 0.1 mg/m^3 cupric sulfate	26

TABLES

Number		Page
1	Changes in Mortality Rate and Mean Survival Time of CD_1 Mice Exposed for 3 Hr to Cupric Sulfate and Challenged with *Streptococcus* Aerosol	12
2	Changes in Mortality Rate and Mean Survival Time of CD_1 Mice Exposed for 3 Hr to Aluminum Sulfate and Challenged with *Streptococcus* Aerosol	13
3	Changes in Mortality Rate and Mean Survival Time of CD_1 Mice Exposed to 3 Hr to Aluminum Ammonium Sulfate and Challenged with *Streptococcus* Aerosol	15
4	Changes in Mortality Rate and Mean Survival Time of CD_1 Mice Exposed to Cupric Sulfate 3 Hr/Day, 5 Day/Week, for 1 or 2 Weeks and Challenged with *Streptococcus* Aerosol	16
5	Effect of Single 3-Hr Exposure to Sulfate Aerosols on Pulmonary Free Cells and Pulmonary Bactericidal Activity	17
6	Effect of Multiple Daily 3-Hr Exposures to Cupric Sulfate Aerosol on Pulmonary Free Cells and Bactericidal Activity	19
7	Cilia Beating Frequency and Percent Normal Epithelium in Hamster and Mouse Trachea Immediately after a 3-Hr Exposure to Sulfates	20
8	Cilia Beating Frequency and Percent Normal Epithelium in Hamster and Mouse Trachea Immediately after 5 and 10 3-Hr Exposures to Cupric Sulfate	22
9	Summary of the Health Effects of Single 3-Hr Exposures to Various Sulfates	28
10	Summary of the Health Effects of Multiple 3-Hr Exposures to Cupric Sulfates	30

LIST OF ABBREVIATIONS

ATP	-- adenosine triphosphate
DMSO	-- dimethylsulfoxide
HEPA	-- high efficiency particulate air filter
MMAD	-- mass median aerodynamic diameter

SECTION 1

INTRODUCTION

As part of its mission, the Environmental Protection Agency is required to assess the toxicity of environmental pollutants whose health effects are not well characterized, or unknown. One possible approach which would enable a rapid response would be the development of a battery of tests which could provide a toxicologic spectrum for the pollutants. The studies described here were designed to determine the ability of selected health effects parameters to provide accurate toxicity data on three particulate air pollutants: cupric sulfate, aluminum sulfate, and aluminum ammonium sulfate. The effects were studied after single 3-hr exposure to all three sulfates or multiple 5 and 10 daily exposures to cupric sulfate. The health-effects parameters included changes in resistance to respiratory infection; pulmonary bactericidal activity; number, type, viability, and adenosine triphosphate (ATP) content of free pulmonary cells; tracheal ciliary beating frequency and morphology; and morphology of the pulmonary system as observed by scanning electron microscopy. The data were correlated to determine which parameters were the most sensitive indicators of toxic effects of the sulfates.

Results of the studies were summarized in a series of quarterly progress reports (IITRI Reports L6107-1, -2, and -3).

SECTION 2

CONCLUSIONS

The results of the studies indicated that changes in mortality rates and mean survival time after respiratory challenge with airborne *Streptococcus pyogenes* were the most consistent and sensitive indicators of damage due to inhalation of sulfate aerosols. The other health effect parameters produced evidence of damage but generally only at the higher pollutant concentrations. Cupric sulfate was the most toxic of the three sulfates but relative toxicity of aluminum sulfate and aluminum ammonium sulfate could not be clearly established. A number of apparent between-sex differences were found, but the differences were inconsistent and probably not related to the sex of the mice.

SECTION 3

RECOMMENDATIONS

Based on the results of this study it is apparent that changes in mortality of mice infected with *S. pyogenes* are the most sensitive indicators of the effects of exposure to metallic sulfates. Therefore future studies should focus on further definition of this assay in laboratory animals other than mice, thus enabling the ranking of toxicity of a given compound in several experimental hosts. Future studies also should include the evaluation of the effects of inhaled metallic sulfates on the immune response. This includes both the cell-mediated as well as humoral immunity.

Results of this study showed that the tracheal epithelium of the CD1 mouse was less suitable for evaluation of sulfates than was that of the hamster. As a result, hamsters rather than mice, should be used for this parameter test.

SECTION 4

MATERIALS AND METHODS

ANIMALS

The 3- to 4-week old male and female CD_1 mice (Charles River Breeding Laboratories, Portage, MI) and the 4-week old male Syrian golden hamsters (Ela:ENG(Syr), ARS/Sprague-Dawley, Madison, WI) were held in quarantine in plastic shoe box cages for 7 to 14 days prior to the initiation of experiments. During exposures to the pollutants and the infectious challenge, the animals were held in special stainless steel wire-mesh cages which held up to 24 animals in individual compartments. Between the intermittent exposures and during the post exposure observation period the animals were housed in plastic shoe box cages. Feed (Rodent Laboratory Chow 5001, Ralston Purina Co., St. Louis, MO) and water were freely available except during exposure periods.

INHALATION EXPOSURES

Exposures to the air pollutants were carried out in 432 liter (120x60x60 cm) Plexiglas chambers supplied with filtered room air. Air temperature and relative humidity were monitored by a thermometer and relative humidity indicator (Model 605, Aireguide Instrument Co., Chicago, IL) mounted in each chamber. During the exposures the temperature ranged from 23 to 30°C and the humidity from 30 to 50% RH. Deotized cage boards (Upjohn Co., Kalamazoo, MI) were placed on the floor of the chambers to prevent ammonia build-up during the exposure periods.

POLLUTANTS

The aerosols were generated by means of a nebulizer (Model 40, DeVilbiss Co., Somerset, PA) containing a 1% (w/v) aqueous solutions of

- cupric sulfate, 102.3% purity ($CuSO_4$, J. T. Baker Chem. Co., Phillipsburg, NJ)
- aluminum sulfate, 99.8% purity ($Al_2(SO_4)_3 \cdot 18H_2O$), Fisher Scientific Co., Fair Lawn, NJ)
- aluminum ammonium sulfate, 99.9% purity ($Al_2(SO_4)_3(NH_4)_2 SO_4 \cdot 24H_2O$, Fisher Scientific Co)

The sulfate solutions were replenished at 30 min intervals during the 3-hr exposure.

The compressed air used to generate and to dilute the aerosol was passed through an oil filter (Model 170, Deltech Engineering, Inc., New Castle, DE) and an air purifier-flow equalizer (Junior Model, Koby Inc., Marlboro, MA). The flow rates of the compressed air to the nebulizer and the dilution vessel were controlled by needle valves equipped with pressure gauges and tube-float flowmeters. The neck of the nebulizer was connected to a 91 cm long valcor glass tube. The first 25 cm of the tube was wrapped with a heating tape controlled by a variable autotransformer (Powerstat, Type 116R, The Superior Electric Co., Bristol, CT) operated at 140 v.

The mass sulfate concentration in the aerosol was determined from two parallel samples collected over the 3-hr exposure period on a 0.2 μm membrane filters (Type GS, 47 mm, Millipore Corp., Bedford, MA). Collection flow rates were controlled by 2.0 liter/min critical orifices previously calibrated with a wet-test meter. The sulfate collected on the filters was assayed by a barium sulfate turbidimetric method and expressed in mg/m^3 of the sulfate. The average of the two samples was used to estimate the concentration of the sulfate in the chamber.

Particle size was determined with an 8-stage cascade impactor (ITRI, Albuquerque, NM) only for the 2.16 mg/m^3 aluminum ammonium sulfate aerosol. The amount of sulfate on each impactor stage was assayed by the barium sulfate turbidimetric method and used to determine the mass median aerodynamic diameter (MMAD) and geometric standard deviation (σg) of the aerosol. For this exposure the MMAD was 0.75 μm with a σg of 2.00. Although particle size measurements were not made during other acute exposures, the aerosol generation and delivery systems were identical to all experiments so that the MMAD and σg were expected to be similar during all exposures. During one multiple exposure to cupric sulfate, aerosol particle size was determined with the quartz crystal microbalance (California Measurements, Sierra Madre, CA); the MMAD was 0.54 μm with a σg of 2.07.

EXPOSURE REGIMENS

The animals were exposed to the experimental atmospheres for a single 3-hr period or in the multiple exposure experiments for 3 hr/day, 5 days/week for 1 or 2 weeks. Since, due to the size of the chambers, it was not possible to expose a sufficient number of animals to measure all experimental parameters on a single day, replicate exposures were carried out on two or more days.

EXPERIMENTAL PARAMETERS

Infectious Challenge

Within 1 hr after the final pollutant exposure, the mice were challenged with an aerosol of *Streptococcus pyogenes* (Lancefield Group C). To maintain the stock culture mice were injected intraperitoneally with a suspension of *S. pyogenes*, killed 24 hr later, and heart blood was incubated on blood agar at 37°C for 48 hr. *S. pyogenes* colonies were isolated and inoculated on blood agar plates with Todd Hewitt broth base (BBL, Cockeysville, MD). After 24 to 48 hr incubation at 37°C, the bacteria were harvested into nutrient broth (Difco Laboratories, Detroit, MI) with added 15% (v/v) glycerol and 1 ml aliquots were frozen at -70°C.

For aerosol dissemination, 0.8 ml from a thawed aliquot was inoculated into 100 ml Todd Hewitt broth, incubated at 37°C for 18 hr, centrifuged at 12,000 g for 10 min and resuspended in 0.1% (w/v) peptone water. The concentration of the bacterial suspension was adjusted to yield 10% transmittance when read at 440 nm in a Spectronic 20 spectrophotometer (Bausch & Lomb, Inc., Northbrook, IL).

The bacterial suspension was placed in the reservoir of a continuous flow Model 841 DeVilbiss nebulizer which was operated with filtered air at a flow rate of 8 liters/min. The bacterial aerosol was disseminated into a 380 liter Plexiglas chamber installed within a microbiologic safety cabinet. After 5 min equilibration period the mice, housed in the individually compartmented stainless steel wire cages, were placed in the chamber. At the end of the 30 min exposure period, ultraviolet germicidal lamps were turned on and the chamber was purged with clean air for 5 min before the mice were removed.

The inhaled dose of *S. pyogenes* was determined in 4 or 6 mice killed immediately after the infectious challenge. The lungs were removed, weighed, and homogenized in 1.8 ml sterile 0.1% (w/v) peptone water. The suspension was diluted, plated onto blood agar, and the *S. pyogenes* colonies were counted after 48 hr incubation at 37°C. The average inhaled dose was 10^5 organisms per mouse.

After the infectious challenge the mice were housed in stainless steel shoe-box cages (8 per cage) in a clean-air, isolated animal room and held for a 14-day observation period during which deaths were recorded daily. The mean survival time (MST) was calculated from the following equation:

$$MST = \frac{\Sigma(A \cdot B)+(d+L)}{n}$$

where A is the last day on which any individual mouse is alive, B is the number of mice surviving A days, d is the last day of observation, L is the number of mice alive on day d, and n is the number of mice in the experimental group.

Pulmonary Defense Mechanism

Details of the methodology and the design of the radioactive bacterial aerosol exposure chamber have been described by Aranyi (1) and Aranyi et al. (2). A brief summary follows:

Pulmonary Bactericidal Activity--

The intrapulmonary bacterial inactivation was determined in individual mice by a modification of the method of Green and Goldstein (3). After exposure to radiolabeled viable bacteria, the ratio of viable bacterial count to radioactive count in each animal's lung defined the rate at which bacteria were destroyed at a given time after infection.

Following the final exposure to the sulfate aerosols, groups of mice were challenged with ^{35}S-labeled *Klebsiella pneumoniae* aerosols. Immediately (0 hr) and at 3 hr after the infectious challenge, 5 (0 hr) and 12 (3 hr) male and female mice, respectively, from each treatment group were killed and their lungs were removed aseptically and homogenized. The total number of bacteria deposited in individual mouse lungs was calculated from radioactive counts of aliquots of the homogenate and the radioactive labeling ratio was determined for each bacterial culture in 0-hr lung samples. Based on these calculations another aliquot of each lung homogenate was appropriately diluted to determine by plate count technique the viable bacteria that remained in the lungs.

The chamber for exposure to the radioactive bacterial aerosol was installed in a glove box. To prevent radioactive and infectious exposure hazard all air exhausted from the system was first passed through an absolute filter placed within the glove box and then through a HEPA filter located on the outside.

The aerosol was produced by a disposable nebulizer (Model X-70, Retec, Cavitron KDC, Inc., Anaheim, CA) operated by compressed air passed through a filter system. Airflow to the nebulizer was regulated by a flowmeter and passed through an electric solenoid valve controlled by a timer. Secondary dilution air provided an adequate air-flow to maintain uniform air temperature and humidity during the aerosol exposure.

Cultures of ^{35}S-labeled *K. pneumoniae* were grown in Anderson's medium in which the magnesium sulfate was replaced by magnesium chloride and the sulfate requirement of the bacteria was provided by addition of ^{35}S-labeled sodium sulfate. After harvest, the bacteria were washed and centrifuged to remove unattached radiolabel. Bacterial counts were initially determined by dark field microscopy with a Petroff-Hausser bacterial counting chamber and later by agar plate culture technique. Radioactive counts were measured with a Mark III Liquid Scintillation System (Model 6880, Searle Analytic Inc., Des Plaines, IL) using samples channels ratio to compute counting efficiency.

Pulmonary Free Cell Population--

Groups of 12 male and female mice were killed within 1 or 24 hr after the final pollutant exposure by intraperitoneal injection of sodium pentobarbital and the lung-heart-trachea complex was removed from the chest *in toto*. A blunted hypodermic needle inserted into the trachea and fastened with suture material was used to lavage the lungs by repeated infusions of warm saline.

The pulmonary cells were isolated from the lavage fluids by centrifugation and washing in Hanks medium. Total cell counts were made using a hemocytometer. Percent distribution of alveolar macrophages, polymorphonuclear leukocytes and lymphocytes were determined by differential counts made on smears of cells obtained by centrifugation, fixed in methanol, and stained with Wright's stain. Viability was determined by dye exclusion using 1% trypan blue.

Cellular adenosine triphosphate (ATP) concentrations in the lavaged cells were determined with a Luminescence Biometer (Model 760, DuPont, Wilmington, DE). For this assay a microsample containing ATP was injected into a suitably buffered reaction mixture of luciferase and luciferin. The peak intensity of the resultant flash of light is directly proportional to the ATP concentration. ATP was extracted by dimethyl sulfoxide (DMSO) from the cells in aliquots of the lavaged and washed cellular suspensions. The extracts were diluted with 0.01 M morpholinopropane sulfonic acid buffer to overcome the quench effect of the high concentration of DMSO in the aqueous extract. ATP levels were expressed as femtograms (10^{-15}g) of ATP per 10^5 cells (calculated from initial cell counts).

Tracheal Explants

Following exposure to a pollutant, four mice and four hamsters were anesthetized by injecting 0.5 cc Brevital sodium (Eli Lilly and Co., Indianapolis, IN). The animals were opened from the middle of the sternum to the larynx and the tracheas were excised, cutting just above the larynx and just above the bifurcation. The tracheas were rinsed in Hanks BSS and then opened from the larynx to the cornia along the membraneous dorsal wall. With a sterile scalpel the trachea was cut transversely into rings approximately 1 mm thick. The tracheal rings were again washed in Hanks BSS and then placed in 35 x 10 mm plastic petri dishes containing 0.7 ml of CMRL 1055 medium supplemented with 0.2% bovine albumin, penicillin (250 U/ml), and streptomycin (250 µg/ml).

Immediately after removal from the animals, tracheal explants were examined using an inverted microscope at 200x. The toxic effects resulting from the exposure to test compounds were expressed as alterations in ciliary activity and cytopathology. Cilia beating frequency at the periphery of each ring explant was determined using an electronic stroboscope (Type 1531-AB, Gen Radio, Concord, MA) as the light source. Cilia beating frequency was measured at each quadrant of the lumen and their average recorded as beats/minute (bpm). The percentage of normal epithelium,

i.e., a smooth luminal surface with beating cilia, was determined for each tracheal ring.

For histopathology studies, tracheal ring explants removed from the animals were immediately fixed in neutral buffered formalin, dehydrated, cleared, and embedded in paraffin and 5 μm sections were cut and stained with hematoxylin and eosin. Tracheal specimens for scanning electron microscopic examination were fixed with Karnovsky's paraformaldehyde-glutaraldehyde phosphate-buffered fixative, critical point dried and coated with gold.

Scanning Electron Microscopy (SEM)

For SEM examination, four mice (two per sex) per treatment were anesthetized by intraperitoneal injection of sodium pentobarbital. The abdomen of each mouse was opened on the midline and the ventral aorta was severed. Following exsanguination the thoracic cavity was opened and the lungs and trachea were removed *en masse*. The trachea was cannulated to the level of the first cartilaginous ring and the lungs were expanded with Karnovsky's paraformaldehyde-glutaraldehyde phosphate-buffered fixative. The trachea was then ligated and the lungs were floated in fixative. The trachea and main-stem bronchi were isolated from the lungs which were then sectioned with a razor blade to reveal the bronchial and alveolar regions of each lung.

Following tracheal cannulation, the head was removed and its skin was retracted. The lower jaw was then removed by sectioning through the ramus. The cranium was excised immediately posterior to the orbits, leaving only the nasal cavity. The nasal bones were reflected with small forceps and the cavity was bisected by inserting a razor blade on one side of the median septum and severing the hard palate.

All tissues were washed in distilled water and dehydrated with increasing concentration of alcohol. Pentyl acetate was then substituted for the alcohol, and the tissues were critical-point dried in carbon dioxide. The dried trachea was sectioned longitudinally and all tissues were cemented to stubs which were gold coated in a vacuum evaporator and examined with a scanning electron microscope (Mark II Stereoscan, Cambridge Instrument Co., London, England) at 20 kV.

STATISTICAL ANALYSES

The Chi-square (X^2) test with 2 x 2 contingency table was used to test hypotheses regarding mortality rates. Student's t test was used to determine the statistical significance of treatment differences in mean survival time, pulmonary bactericidal activity, and pulmonary free cell parameters. Hypotheses involving cilia beating frequency were tested with two-way analysis of variance. Duncan's New Multiple-Range Test was used to detect treatment differences. Treatment differences in percent normal epithelium were determined from the Chi-square distribution. Unless indicated otherwise the significance of treatment differences was reported at the $p<0.05$ probability level.

Statistical analysis of the mortality rate data was conducted also with log-linear modeling procedures (4,5) which provide maximum likelihood estimates, standard errors, and tests of significance for the main effects and interactions of discrete or qualitative data, such as mortality. Log-linear models containing terms that relate the dose-response to differences between control and pollutant-exposed mice (linear pollutant concentration x experimental groups interaction) and to differences between sexes (linear pollutant concentration x experimental group x sex interaction) were fitted to the mortality rate data. In the multiple-exposure to cupric sulfate experiments, a term for number of exposures was substituted for the pollutant concentration term and a corresponding log-linear model was fitted to the mortality data.

SECTION 5

RESULTS

Air pollutant concentrations for the single 3-hr exposures were selected to provide a meaningful dose reponse for each pollutant. Accordingly, as appropriate, data from individual exposures have been combined to provide a "high", "intermediate", and "low" concentration for each pollutant. For the three test compounds the actual mean concentrations of sulfate (in mg/m^3) during the 3-hr exposures were as follows:

	High		Intermediate		Low	
	Mean	Range	Mean	Range	Mean	Range
Cupric sulfate	2.53	2.41-2.64	0.93	0.75-1.13	0.43	
Aluminum sulfate	2.71	2.66-2.75	2.31	2.26-2.36	1.84	1.65-2.02
Aluminum ammonium sulfate	3.79	3.76-3.81	2.10	1.95-2.25	1.47	

MORTALITY

A single 3-hr exposure to an aerosol of cupric sulfate at the various test concentrations significantly ($p<0.001$) increased the mortality rate and decreased the mean survival time after challenge with *S. pyogenes* in both male and female mice (Table 1). The magnitude of the response appeared to be directly related to the sulfate concentration

Analysis of the single exposure cupric sulfate data by log-linear modeling revealed a highly significant ($p<0.001$) dose-response relationship; i.e., there were linearly increasing differences in mortality between the control and pollutant-exposed mice with increasing sulfate concentration. There was a significant ($p<0.05$) between-sex difference in overall mortality, but there was no sex-related difference in the dose-response relationship. The increasing difference in mortality between control and pollutant-exposed mice over the sulfate concentrations was the same for both sexes. This suggests that the response to the pollutant is not related to the severity of the infectious challenge.

Table 2 shows the mortality rates and mean survival times of mice exposed for 3 hr to aerosols of aluminum sulfate and then challenged with the infectious aerosol. Exposure to 2.71 mg/m^3 aluminum sulfate significantly increased the mortality rate and decreased the survival time for both the male and female mice. After exposure to 2.31 mg/m^3 aluminum sulfate the mortality rate among the female mice increased and survival time decreased significantly; these changes were not seen in the male mice. Data from the lowest concentration of aluminum sulfate (1.84 mg/m^3) were similar to the intermediate, but the differences were not significant, possibly due to the fewer number of mice used in these experiments.

TABLE 1. CHANGES IN MORTALITY RATE AND MEAN SURVIVAL TIME OF CD1 MICE EXPOSED FOR 3 HR TO CUPRIC SULFATE AND CHALLENGED WITH *STREPTOCOCCUS* AEROSOL

SO_4 mg/m^3	Sex	No. of Mice		Change[a]	
		Con.	Exp.	Mortality %	Mean Survival Time, days
2.53	M	24	23	+100***	-11.0***
	F	24	24	+100***	-11.0***
0.93	M	98	100	+61.7***	-4.9***
	F	97	100	+77.6***	-6.7***
0.43	M	24	24	+54.2***	-4.2***
	F	24	23	+70.3***	-5.9***

[a] Sulfate exposed - control animals

*** $p<0.001$

TABLE 2. CHANGES IN MORTALITY RATE AND MEAN SURVIVAL TIME OF CD_1 MICE EXPOSED FOR 3 HR TO ALUMINUM SULFATE AND CHALLENGED WITH *STREPTOCOCCUS* AEROSOL

SO_4 mg/m^3	Sex	No. of Mice		Change[a]	
		Con.	Exp.	Mortality %	Mean Survival Time, days
2.71	M	57	56	+18.1*	-1.5**
	F	57	54	+39.6***	-3.5***
2.31	M	47	61	+4.8	-0.9
	F	48	62	+18.6*	-1.4*
1.84	M	24	24	+4.1	-1.0
	F	24	24	+20.8	-1.8

[a] Sulfate exposed - control animals

* $p<0.05$

** $p<0.01$

*** $p<0.001$

Results of the log-linear analysis of the aluminum sulfate paralleled those with cupric sulfate. There was a significant ($p<0.05$) dose-response relationship and an overall between-sex difference in mortality, but again there was no sex difference in the dose-response.

Changes in mortality rate and mean survival time of mice exposed for 3 hr to aluminum ammonium sulfate and challenged with the infectious agent are shown in Table 3. The mortality rate increased and MST decreased in all groups of mice exposed to the pollutants. However, difference between treatment groups was significant only in the intermediate exposure group (2.10 mg/m^3) where female mice showed an increased mortality rate ($p<0.01$) and reduced mean survival time ($p<0.001$).

When the aluminum ammonium sulfate data were analyzed with log-linear model, the only significant effect on mortality was a main effect of experimental group, i.e., the pollutant-exposed mice had higher mortality when averaged over both sexes and the three concentrations. There was no linear dose-response and there were no between-sex differences.

In the multiple exposure studies (Table 4) no changes were observed when mice were challenged with *S. pyogenes* aerosol following five daily 3-hr exposures to approximately 0.1 mg/m^3 cupric sulfate. However, mice challenged with the infectious agent after 10 daily exposures had significantly increased mortality rate and decreased mean survival time.

Similarly, in the log-linear analysis, mortality was significantly increased ($p<0.05$) with increased exposure to cupric sulfate. There were no between-sex differences in mortality.

PULMONARY DEFENSE MECHANISMS

Table 5 shows the effects of a single 3-hr exposure to various sulfates on pulmonary bactericidal activity and pulmonary free cell population.

Pulmonary Free Cells

Within 1 hr of a single exposure to 2.53 mg/m^3 cupric sulfate, the only significant change observed in pulmonary free cell population was a decrease in total cell count in the male mice. However 24 hr after the exposure total cell counts were significantly reduced and cellular ATP levels were significantly elevated in lung lavages obtained from male and female mice. At the intermediate concentrations of cupric sulfate (0.93 mg/m^3) no significant differences were detected.

After exposure to 2.71 mg/m^3 of aluminum sulfate total cell count was significantly reduced in female, but not male mice within 1 hr after exposure and in both sexes 24 hr after the exposure. The cellular ATP levels were not affected. At the lower aluminum sulfate concentration (1.84 mg/m^3) no differences in pulmonary free cells were seen when measured within 1 hr following exposure.

TABLE 3. CHANGES IN MORTALITY RATE AND MEAN SURVIVAL TIME OF CD_1 MICE EXPOSED FOR 3 HR TO ALUMINUM AMMONIUM SULFATE AND CHALLENGED WITH *STREPTOCOCCUS* AEROSOL

SO_4 mg/m^3	Sex	No. of Mice		Change[a]	
		Con.	Exp.	Mortality %	Mean Survival Time, days
3.79	M	24	24	+16.7	-1.3
	F	24	23	+13.2	-1.2
2.10	M	48	49	+5.2	-0.8
	F	48	50	+27.0**	-2.3***
1.47	M	24	24	+16.7	-1.5
	F	24	24	+8.3	-1.0

[a] Sulfate exposed - control animals

* $p<0.05$

** $p<0.01$

***$p<0.001$

TABLE 4. CHANGES IN MORTALITY RATE AND MEAN SURVIVAL TIME OF CD_1 MICE EXPOSED TO CUPRIC SULFATE 3 HR/DAY, 5 DAY/WEEK, FOR 1 OR 2 WEEKS AND CHALLENGED WITH *STREPTOCOCCUS* AEROSOL

No. of Exposures	SO_4 mg/m^3 Mean	SD	Sex	No. of Mice Con.	Exp.	Change[a] Mortality %	Mean Survival Time, days
5	0.09	0.01	M	48	47	-3.9	+0.5
			F	48	48	-2.1	+0.1
10	0.10	0.02	M	48	48	+31.2**	-1.54*
			F	48	48	+25.0**	-1.29*

[a] Sulfate exposed - control

* $p<0.05$

** $p<0.01$

*** $p<0.001$

TABLE 5. EFFECT OF SINGLE 3-HR EXPOSURE TO SULFATE AEROSOLS ON PULMONARY FREE CELLS AND PULMONARY BACTERICIDAL ACTIVITY

Pollutant	SO_4 mg/m^3	Interval Exposure/ Assay hr	Sex	Pulmonary Free Cells: Total Cells (Count x10^5) n(1)	Total Cells %(2)	Cellular ATP (10^8fg/10^5 cells) n(1)	Cellular ATP %(2)	Bactericidal Activity: *K. pneumoniae* killed (%) n(1)	*K. pneumoniae* killed (%) %(2)
Cupric Sulfate	2.53	<1	M	26	42.0***	20	74.6	24	61.0***
			F	25	79.0	23	83.5	24	57.1***
		24	M	24	36.5***	23	144.0***		N.D.
			F	24	60.6**	21	181.0**		N.D.
	0.93	<1	M	38	98.0	33	113.5	44	98.8
			F	36	92.4	30	102.4	44	97.5
		24	M	36	88.5	31	105.9		N.D.
			F		NOT DONE				N.D.
Aluminum Sulfate	2.71	<1	M	16	127.7	16	96.6	44	104.8**
			F	18	73.5*	16	102.2	47	113.6***
		24	M	34	81.0*	30	116.4		N.D.
			F	34	80.7*	32	97.8		N.D.
	1.84	<1	M	16	65.4	16	95.2	23	100.5
			F	16	105.8	16	90.2	23	96.6
Aluminum Ammonium Sulfate	3.79	<1	M	16	79.3	15	170.1***		N.D.
			F	16	122.6	16	213.6***		N.D.
	2.10	<1	M	16	200.9	16	89.3	20	127.7***
			F	16	111.7	16	121.6	18	113.1*
		24	M	32	84.3	31	79.8*		N.D.
			F	32	126.8	32	92.0		N.D.

(1) number of mice used

(2) percent of control

N.D. not done

Significant change from corresponding control mice exposed to filtered air

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

No significant changes in total cell counts were detected in mice exposed to aluminum ammonium sulfate. Both male and female mice showed significantly increased pulmonary cellular ATP levels after exposure to 3.79 mg/m^3 of aluminum ammonium sulfate. The ATP levels were significantly reduced in cells lavaged from lungs of male mice 24 hr after but not within 1 hr after exposure to 2.10 mg/m^3 aluminum ammonium sulfate.

Multiple exposures to cupric sulfate aerosols (Table 6) did not result in significant changes in total count or ATP levels of pulmonary free cells. Similarily, differential counts and viability of the pulmonary free cells, not shown in the table, were not affected by any of the exposure conditions. Generally, viability was more than 96% and approximately 98% of the cells were alveolar macrophages.

Bactericidal Activity

Pulmonary bactericidal activity (Table 5) was significantly reduced in both male and female mice exposed for 3-hr to 2.53 mg/m^3 cupric sulfate, but was not affected by exposure to the lower concentration (0.93 mg/m^3). Exposure to 2.71 mg/m^3 aluminum sulfate resulted in a significant increase of bactericidal activity in both sexes of mice. However, no changes after exposure to 1.84 mg/m^3 aluminum sulfate were observed. The pulmonary bactericidal activity was also significantly increased in mice exposed to 2.10 mg/m^3 aluminum ammonium sulfate aerosol.

Multiple exposures to cupric sulfate aerosols resulted in significantly decreased bactericidal activity in female mice after 5 daily 3-hr exposures, and in both female and male mice after 10 daily exposures (Table 6).

TRACHEAL EPITHELIUM

The effects of single 3-hr exposure to various sulfate aerosols on cilia beating frequency and percent normal epithelium are shown in Table 7. No differences in response were seen between the male and female mice, thus the data from both sexes were combined for statistical analyses.

Significant ($p < 0.05$) decrease in cilia beating frequency was seen only in hamsters exposed for 3 hr to 2.53 mg/m^3 cupric sulfate or 3.79 mg/m^3 aluminum ammonium sulfate. Cytologic alterations were observed in the tracheal epithelium of hamsters exposed to the sulfates. In control hamsters the mean percent of normal epithelium, defined as a smooth luminal surface with beating cilia, ranged from 62 to 93%. Significant decreases in the normal epithelium was seen in hamsters after exposure to 2.53 mg/m^3 cupric sulfate, 1.84 and 2.71 mg/m^3 aluminum sulfate and 3.79 mg/m^3 aluminum ammonium sulfate. No changes in percent of normal epithelium were observed in mice which suggests that hamster tracheal epithelium is more sensitive to exposure to sulfates than that of the mouse.

Microscopic examination of tracheas from mice after a 3-hr exposure to the sulfate aerosol showed epithelium similiar to those of control mice exposed to filtered air. An observation common to control mice and those exposed to sulfate was exfoliation of differentiated surface epithelial

TABLE 6. EFFECT OF MULTIPLE DAILY 3-HR EXPOSURES TO CUPRIC SULFATE AEROSOL ON PULMONARY FREE CELLS AND BACTERICIDAL ACTIVITY

Exposure				Pulmonary Free Cells				Bactericidal Activity	
				Total Cells (Count x10^5)		Cellular ATP (10^6fg/10^5 cells)		*K. pneumoniae* killed (%)	
SO_4 mg/m^3 Mean	SD	Number of Exposures 3 hr/day	Sex	n(1)	%(2)	n(1)	%(2)	n(1)	%(2)
0.09	0.01	5	M	17	100.6	15	86.5		
0.09	0.01	5	F	17	71.1	16	92.9	NOT DONE	
0.09	0.03	5	M					22	102.0
0.09	0.03	5	F	N O T D O N E				24	94.2*
0.10	0.02	10	M	16	75.8	15	91.3	22	94.5*
0.10	0.02	10	F	17	82.5	17	101.8	18	85.2**

(1) number of mice used

(2) percent of control

Significant change from corresponding control mice exposed to filtered air
* $p<0.05$ ** $p<0.01$

TABLE 7. CILIA BEATING FREQUENCY AND PERCENT NORMAL EPITHELIUM IN HAMSTER AND MOUSE TRACHEA IMMEDIATELY AFTER A 3-HR EXPOSURE TO SULFATES

Pollutant	SO_4 mg/m^3	Mean Cilia Beat/min[a]				Normal Epithelium,%[b]			
		Mouse		Hamster		Mouse		Hamster	
		Air	SO_4	Air	SO_4	Air	SO_4	Air	SO_4
Cupric Sulfate	2.53	1370	1359	1248	1153*	62	60	75	51*
	0.93	1306	1293	1216	1225	55	49	62	56
Aluminum Sulfate	2.71	1396	1388	1222	1209	-	-	80	55*
	1.84	N.D.	N.D.	1256	1203	N.D.	N.D.	91	75*
Aluminum Ammonium Sulfate	3.79	N.D.	N.D.	1216	1143*	N.D.	N.D.	93	59*
	2.10	1322	1320	1261	1231	77	65	93	86

[a] Mean of 48 observations of 12 tracheal rings from 4 animals.

[b] Mean of 12 tracheal rings from 4 animals. Normal epithelium is defined as a smooth surface with beating cilia.

* Significant difference from control animals exposed to filtered air ($p<0.05$).

- Bubbling of epithelium with debris present; Cilia movement was visible and appeared normal, but quantitative evaluation could not be made.

N.D. not done

cells from the mucosa. However, in mice exposed to sulfates the percentage of desquamation was greater. Cytoplasmic vacuolation was seen in approximately 10% of the epithelium after exposure to the sulfates. Tracheas from control hamsters showed a surface epithelium of normal pseudostratified ciliated columnar appearance with occasional loss of cilia. After exposure to cupric or aluminum sulfate approximately 75% of the tracheas had epithelium similar to their controls. Moderate epithelial flattening and focal areas of exfoliation of surface epithelial cells as well as focal epithelial ulceration was seen in some specimens.

Upon scanning electron microscopic examination tracheal epithelial surface consists of three distinct cell types, namely ciliated, microvillous, and goblet or mucus-secreting. Tracheas from control hamsters and mice, had knob-like structures on the distal end of the microvilli and fully formed ciliated cells. Patchy and isolated areas of unciliated cells were seen in the hamsters, while tracheal tissue of the control mice was more uniform in appearance. Hamster tracheal epithelium removed and fixed immediately after exposure to the higher level of cupric or aluminum ammonium sulfate showed increased amounts of mucus covering the surface of the microvillous cells. The epithelial surfaces of tracheas from mice exposed to sulfates were unchanged.

Table 8 shows the effects of multiple 3-hr daily exposures to cupric sulfate on the tracheal epithelium. Following five exposures to the sulfate aerosol, male mice showed a significant reduction ($p < 0.05$) in the percent of normal epithelium compared to the controls. However, the relatively small amount ($\sim$40%) of normal epithelium seen in the control mice makes the change of questionable practical significance. After 10 exposures to cupric sulfate, female but not male mice showed a reduced ($p < 0.05$) percent normal epithelium. In these mice 25% of the epithelial surface had a jagged appearance due to exfoliating cells.

No changes in cilia beat frequency were observed in hamsters after 5 or 10 exposures. Cytologic and histologic examination of the tracheal mucosa from the hamsters revealed no treatment-related alterations. Both control and sulfate-exposed hamsters had normal pseudostratified ciliated columnar epithelium. An occasional mucous cyst was observed in hamsters exposed 10 times to the cupric sulfate. In mice,bubbling of the epithelium and the presence of debris on the mucosal surface prevented the evaluation of this parameter. Thus, a significant finding in these studies was the poor quality of the respiratory epithelium in control mice. Because of large areas of cellular necrosis with loss of cilia and desquamation, the tracheas of the CD_1 mice do not appear to be appropriate for the assessment of air pollutant effects.

SCANNING ELECTRON MICROSCOPY

Upon scanning electron microscopic examination mice exposed for 3 hr to 2.53 mg/m^3 of cupric sulfate had crystalline particles scattered throughout the respiratory system (Figure 1a,b,c,d). In the nasal cavities, particles were found on both ciliated and nonciliated cells. A heavy mucous layer was noted in the trachea. The crystalline particles were observed in areas of the trachea where no mucus was present. Most of the particles were attached to or near ciliated cells (Figure 1a).

TABLE 8. CILIA BEATING FREQUENCY AND PERCENT NORMAL EPITHELIUM IN HAMSTER AND MOUSE TRACHEA IMMEDIATELY AFTER 5 AND 10 3-HR EXPOSURES TO CUPRIC SULFATE

No. of Exposures	SO_4 mg/m^3 Mean	SD	Cilia Beat/min[a] Hamster[c]	Normal Epithelium, %[b] Mouse Male	Mouse Female	Hamster
5	0 (Filtered Air)		1263	44	36	98
	0.09	0.01	1213	30*	28	89
10	0 (Filtered Air)		1227	73	83	97
	0.10	0.02	1190	71	73*	85

[a] Mean of 48 observations of 12 trachela rings from 4 animals.

[b] Mean of 12 tracheal rings from 4 animals. Normal epithelium is defined as a smooth surface with beating cilia.

[c] Cilia beat in mouse organ culture could not be evaluated due to bubbling of epithelium.

* Significant difference from control animals exposed to filtered air ($p<0.05$).

Figure 1. Respiratory tract of mouse exposed to 2.53 mg/m^3 cupric sulfate for 3 hr. Crystalline particles are found throughout the system: a) trachea 4000X, b) bronchus 5750, c) terminal bronchiole 9000X and d) alveoli 4500X.

Within the lung, particles were observed in the bronchi (Figure 1b), terminal bronchioles (Figure 1c) and alveoli (Figure 1d). No cellular destruction was observed, but this was expected since the tissues were obtained shortly after exposure to the pollutant.

Some debris was seen in the respiratory tracts of mice exposed to 0.93 mg/m^3 cupric sulfate but much less than that seen at the higher concentration.

Neither particles nor cellular damage were observed in the respiratory tracts of mice exposed to 2.71 mg/m^3 aluminum sulfate. However, rod-like particles were seen in the brochial (Figure 2a)) and alveolar (Figure 2b) regions of the lungs of mice exposed to 2.10 mg/m^3 aluminum ammonium sulfate. As in the cupric sulfate study, no cellular destruction was observed.

In the multiple exposure studies all control mice showed generally normal respiratory tract structure (Figures 3a and 3b), although occasionally some alveolar thickening was seen at the lung periphery. The bronchi of mice exposed for 5 or 10 times were normal except for the presence of rod-shaped debris (Figure 4). After 5 exposures some alveoli were slightly thickened and irregular in appearance (Figure 5). After 10 exposures to cupric sulfate aerosol the mice showed extensive areas of alveolar thickening with many of the walls fused into irregular masses (Figure 6).

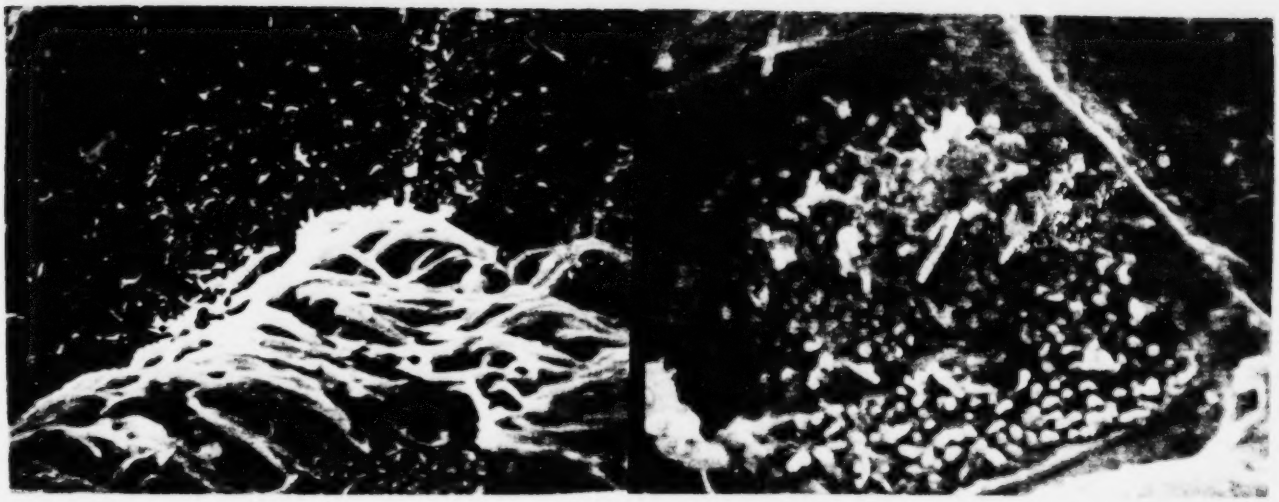


Figure 2. Respiratory tract of mouse exposed to 2.10 mg/m^3 aluminum ammonium sulfate for 3 hr. Rod-like particles are found in a) bronchus 5600X and b) alveoli 11,000X.

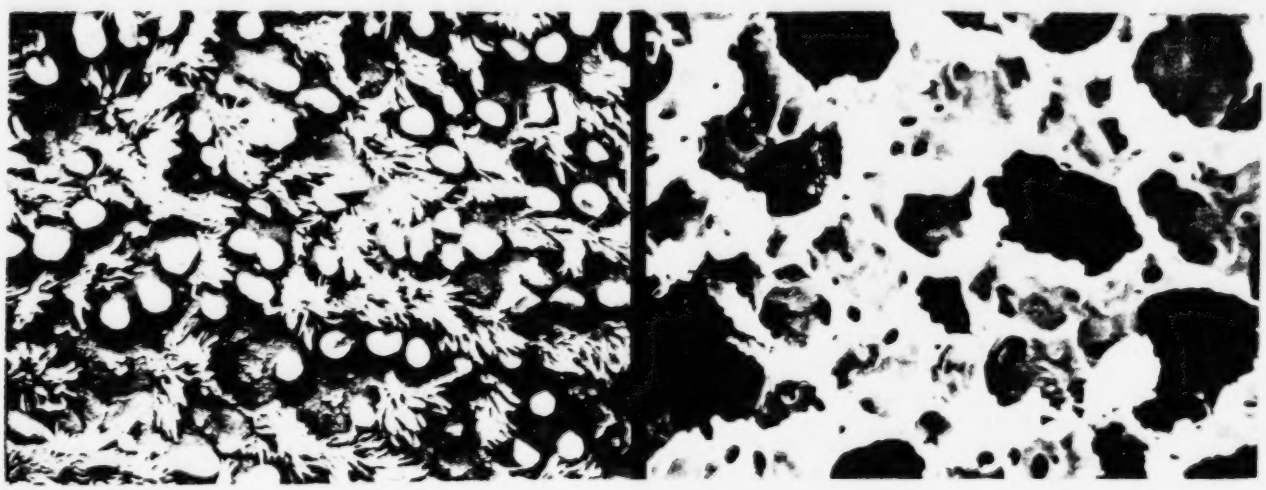
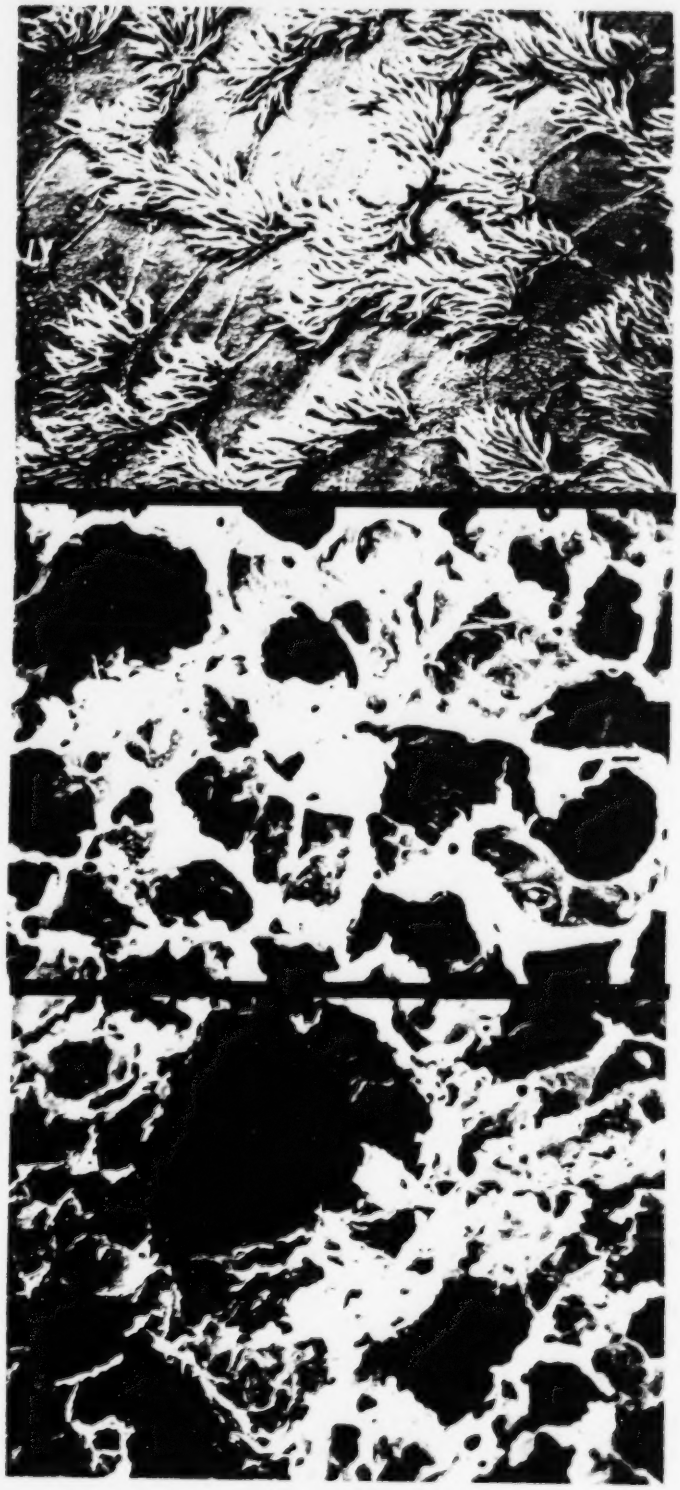


Figure 3. Respiratory tract of control mouse after 5 daily 3-hr exposures to filtered room air: a) bronchus 900X and b) alveoli 475X.

Figure 4. Bronchus of mouse after 10 daily 3-hr exposures to 0.1 mg/m^3 cupric sulfate. Notice the rod-shaped debris. 1050X.

Figure 5. Alveoli of mouse after 5 daily 3-hr exposures to 0.1 mg/m^3 cupric sulfate. Thickening and irregular appearance of the alveoli are found. 475X.

Figure 6. Alveoli of mouse after 10 daily 3-hr exposures to 0.1 mg/m^3 cupric sulfate. The alveoli are thickened with many of the walls fused into irregular masses. 525X.



SECTION 6

DISCUSSION

The quantifiable health effects resulting from the single 3-hr exposure to cupric, aluminum and aluminum ammonium sulfates are summarized in Table 9.

Exposure to all three concentrations of cupric sulfate aerosol produced an increase in the mortality rate and decrease in mean survival time of CD_1 mice challenged with *S. pyogenes*. These results are similar to previous work in our laboratory in which CD_2F_1 mice were exposed to cupric sulfate aerosols for a single 3 hr period (6). Significant changes were also noted in total pulmonary cell count, ATP activity in alveolar macrophages and pulmonary bactericidal activity in mice and in percent normal epithelium in hamsters exposed to the highest concentration of cupric sulfate (2.53 mg/m^3).

With aluminum sulfate there was an apparent dose-related effect on mortality with no changes in mortality at the lower concentrations. Again, these were in agreement with earlier studies with this pollutant (6). Similar effects were seen with the total pulmonary cell counts (1 or 24 hr after exposure) and pulmonary bactericidal activity although there was some between sex variability. At the lower concentration (1.84 mg/m^3) the only significant effect was a decrease in percent of normal tracheal epithelium in hamsters.

Changes in all parameters, except total pulmonary cell count, were observed after exposure to the highest concentration of aluminum ammonium sulfate (3.79 mg/m^3). After exposure to the intermediate concentration (2.10 mg/m^3) there were effects on pulmonary cell ATP content and bactericidal activity in male mice. However, it should be noted that exposures to both aluminum ammonium sulfate and aluminum sulfate resulted in an increase in pulmonary bactericidal activity when compared to control mice exposed to filtered air. In other studies we have frequently observed increased bactericidal activity in lungs of mice exposed to aerosols of pollutants that had no apparent significant effect on the other parameters. However, exposures to aerosols of pollutants that produced detrimental health effects resulted in depressed bactericidal activity. These increases may be due to activation of the alveolar macrophages by having phagocytized the inhaled dust. On the other hand, inhalation of toxic aerosols could overcome this initial activation and then depress the bactericidal capacity of the macrophages in the lungs (1).

TABLE 9. SUMMARY OF THE HEALTH EFFECTS OF SINGLE 3-HR EXPOSURES TO VARIOUS SULFATES

Pollutant	SO_4 mg/m^3	Mortality Rate CD1 M	Mortality Rate CD1 F	Mortality Rate CD1 M&F	MST[a] CD1 M	MST[a] CD1 F	MST[a] CD1 M&F	Total Cells[b] CD1 M	Total Cells[b] CD1 F	Total Cells[c] CD1 M	Total Cells[c] CD1 F	ATP[b] CD1 M	ATP[b] CD1 F	ATP[c] CD1 M	ATP[c] CD1 F	PBA[d] CD1 M	PBA[d] CD1 F	Cilia Beat/Min CD1	Cilia Beat/Min Hamster	Normal Epithelium,% CD1	Normal Epithelium,% Hamster
Cupric Sulfate	2.53	+	+		-	-		-	0	-	-	0	0	+	+	-	-	0	-	0	-
	0.93	+	+		-	-		0	0	0	ND	0	ND	0	ND	0	0	0	0	0	0
	0.43	+	+		-	-		ND		ND		ND		ND		ND		ND	ND	ND	ND
Aluminum Sulfate	2.71	+	+		-	-		0	-	-	-	0	0	0	0	+	+	0	0	e	-
	2.31	0	+		0	-		ND		ND		ND		ND		ND		ND	ND	ND	ND
	1.84	0	0		0	0		0	0	ND		0	0	ND		0	0	ND	0	ND	
Aluminum Ammonium Sulfate	3.79	0	0		0	0		0	0	ND		+	+	ND		ND		ND	-	ND	-
	2.10	0	+		0	-		0	0	0	0	0	0	-	0	+	+	0	0	0	0
	1.47	0	0		0	0		ND		ND		ND		ND		ND		ND	ND	ND	ND

[a] Mean Survival Time

[b] Mice killed within 1 hr after exposure

[c] Mice killed 24 hr after exposure

[d] Pulmonary Bactericidal Activity

[e] Unable to make evaluation due to technical difficulty

\+ Significantly greater than control ($p<0.05$)

\- Significantly lower than control ($p<0.05$)

0 No significant changes

The mortality resulting from exposure to aluminum ammonium sulfate was not in agreement with previous work which suggested that this compound was less toxic than aluminum sulfate (6). In the present study no clear dose response was achieved, but the number of mice used in the studies was relatively small.

From the data it is apparent that mortality rate and mean survival time were the most consistent and sensitive indicators of damage resulting from exposure to the pollutants. However, the size of the exposure chambers limited the number of animals available for each study. Thus, the unavailability of a large number of animals may have reduced the sensitivity of some of the other assays, particularly the pulmonary defense mechanism parameters.

The effects of the multiple 3-hr exposures to 0.1 mg/m^3 cupric sulfate are tabulated in Table 10. After five exposures the only effects were a significant increase in pulmonary bactericidal activity in female mice and a decrease in percent normal epithelium in hamsters. Mortality rate and survival time did not differ significantly from the controls although the total dose of cupric sulfate estimated as a product of concentration x time (1.35 mg/m^3) was equivalent to a dose in the single 3-hr exposure which greatly altered these parameters. After 10 exposures increased mortality rate and decreased pulmonary bactericidal activity was seen in male and female mice and decrease in percent normal epithelium in female mice only. Thus, in the multiple exposure studies the mortality rate again appeared to be the most sensitive indicator of the damage.

Across-pollutant comparison indicated that cupric sulfate was the most toxic of the three sulfates. In earlier studies with CD_2F_1 mice (6), aluminum sulfate was more effective in reducing the resistance to streptococcal infection than was aluminum ammonium sulfate, but in the present studies the differences in toxicity of the two compounds was less evident. While aluminum ammonium sulfate was not as toxic as aluminum sulfate at the higher concentrations, significant increases in mortality rates and decreases in survival time were found at the lower concentrations of aluminum ammonium sulfate, but not aluminum sulfate.

Finally, during the single and multiple 3-hr exposures a number of effects were seen, which appeared only in male or in female mice. However, there did not appear to be any clearly consistent pattern in these responses suggesting that these differences were most probably spurious and not related to the sex of the mouse.

TABLE 10. SUMMARY OF THE HEALTH EFFECTS OF MULTIPLE 3-HR EXPOSURES TO CUPRIC SULFATES

No. of Exposures	SO_4 mg/m^3 Mean	SD	Mortality Rate CD$_1$ M	F	M&F	MST[a] CD$_1$ M	F	M&F	Total Cells[b] CD$_1$ M	F	ATP[b] CD$_1$ M	F	PBA[c] CD$_1$ M	F	Cilia Beat/Min CD$_1$ M	F	Hamster	Normal Epithelium,% CD$_1$ M	F	Hamster
5	0.09	0.01	0	0	0	0	0	0	0	0	0	0			d	d	0	-	0	0
	0.09	0.01											0	-						
10	0.10	0.02	+	+	+	-	-	-	0	0	0	0	-	-	d	d	0	0	-	0

[a] Mean survival time

[b] Mice killed within 1 hr after exposure

[c] Pulmonary Bactericidal Activity

[d] Unable to make evaluation due to technical difficulty

\+ Significantly greater than control ($p<0.05$)

\- Significantly lower than control ($p<0.05$)

0 No significant changes

REFERENCES

1. Aranyi, C. Effects of Industrial Particulate Samples on Alveolar Macrophages. EPA-600/51-81-003. U.S. Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park, NC, 1981.

2. Aranyi, C., J. Bradof, D. E. Gardner and J. Lewtas-Huisingh. In Vitro and In Vivo Evaluation of Potential Toxicity of Industrial Particles. in Short-Term Bioassays in the Analysis of Complex Environmental Mixtures. M. D. Waters, S. S. Sandhy, J. Lewtas-Huisingh, L. Claxton and S. Nesnow, Eds. Plenum Press, New York, NY In Press. 1981.

3. Green, G. M. and E. Goldstein. A Method for Quantitating Intrapulmonary Bacterial Inactivation in Individual Animals. J. Lab. Clin. Med. 68:669-677, 1966.

4. Bishop, Y. M. M., S. E. Fineberg, and P. W. Holland. Discrete Multivariate Analysis:Theory and Practice. MIT Press. Cambridge, MA. 1975.

5. Haberman, S. J. Analysis of Qualitative Data. Academic Press, New York, NY. 1979.

6. Ehrlich, R. Interaction Between Environmental Pollutants and Respiratory Infections. Environ. Hlth. Persp. 35:89-100, 1980.